



Review

New molecular insights into the mechanisms of cholestasis[☆]

Martin Wagner, Gernot Zollner, Michael Trauner*

Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria

Recent progress in basic research has enhanced our understanding of the molecular mechanisms of normal bile secretion and their alterations in cholestasis. Genetic transporter variants contribute to an entire spectrum of cholestatic liver diseases and can cause hereditary cholestatic syndromes or determine susceptibility and disease progression in acquired cholestatic disorders. Cholestasis is associated with complex transcriptional and post-transcriptional alterations of hepatobiliary transporters and enzymes participating in bile formation. Ligand-activated nuclear receptors for bile acids and other biliary compounds play a key role in the regulation of genes required for bile formation. Pharmacological interventions in cholestasis may aim at modulating such novel regulatory pathways. This review will summarize the principles of molecular alterations in cholestasis and will give an overview of potential clinical implications.

© 2009 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Keywords: Cholestasis; Bile acids; Hepatobiliary transporters; Nuclear receptors

1. Introduction

The hallmark of cholestasis is an impairment of bile secretion and flow followed by a lack of bile in the intestine and accumulation of potentially toxic cholephiles in the liver and the systemic circulation [1]. Normal bile

formation depends on the proper function of distinct membrane transport systems along the enterohepatic route, i.e., in hepatocytes, bile duct epithelial cells (cholangiocytes) and enterocytes, and its coordinated transcriptional regulation by nuclear receptors (NRs) as well as posttranscriptional mechanisms [2,3]. Hereditary mutations of transporter genes or exposure to cholestatic injury (e.g., drugs, hormones, pro-inflammatory cytokines and biliary obstruction/destruction) result in reduced expression and function of hepatobiliary transport systems, which play an important role in the pathogenesis of cholestasis. In addition to genetic or acquired transporter changes, other mechanisms such as altered cell polarity, disruption of cell-to-cell junctions and cytoskeletal changes may be involved [1]. Unravelling the molecular mechanism of cholestasis not only extends our understanding of the pathophysiological pathways but also impacts on the clinical management of patients by identifying novel targets for diagnosis, prognosis and pharmacological interventions. This review summarizes the principal molecular mechanisms of cholestasis and their potential clinical significance with a focus on hepatobiliary transport and metabolism of bile acids, bilirubin and other biliary

Associate Editor: F. Lammert

[☆] The authors who have taken part in this study declared that they do not have anything to disclose regarding funding from industry or conflict of interest with respect to this manuscript.

* Corresponding author. Fax: +43 316 3853062.

E-mail address: michael.trauner@meduni-graz.at (M. Trauner).

Abbreviations: ABC, ATP-binding cassette; BSEP, bile salt export pump; BRIC, benign recurrent intrahepatic cholestasis; CAR (NR1I3), constitutive androstane receptor; CBDL, common bile duct ligation; CYP, cytochrome p450; FXR (NR1H4), farnesoid X receptor/bile acid receptor; HNF, hepatocyte nuclear factor; ICP, intrahepatic cholestasis of pregnancy; LPS, lipopolysaccharide; MDR, multidrug resistance gene; MRP, multidrug resistance-associated protein; NTCP (*SLC10A1*), Na⁺/taurocholate cotransporter; OATP (*SLC21A*), organic anion transporter; OST, organic solute transporter; PBC, primary biliary cirrhosis; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis; PXR (NR1I2), pregnane X receptor; RAR α (NR1B1), retinoic acid receptor; RXR α (NR2B1), retinoid X receptor; SHP (NR0B2), short heterodimer partner; TNF α , tumor necrosis factor alpha.

constituents. Progress in the immunopathogenesis of cholangiopathies such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) has recently been summarized elsewhere [4–7].

2. Principal molecular mechanisms and regulation of bile formation

Primary secretion of bile occurs at the level of bile canaliculi of hepatocytes. Canalicular excretion of bile acids, the major fraction of organic solutes in bile, is mediated by ATP-binding cassette (ABC) transporters for bile acids and non-bile acid organic anions and represents the rate limiting step in bile formation [8]. Excretion of osmotically active bile acids is followed by movement of water via aquaporin channels and tight junctions (“bile acid-dependent bile flow”). In addition to their osmotic activity, bile acids promote canalicular secretion of phospholipids and cholesterol for formation of mixed biliary micelles [9]. Canalicular excretion of reduced glu-

tathione and bicarbonate accounts for the major components of the “bile acid-independent” fraction of bile flow [10]. “Canalicular bile” is further modified by secretory and absorptive processes as it passes along bile ductules and ducts which secrete mainly bicarbonate (“ductal bile”) [4,11,12]. Several biliary compounds such as bile acids undergo extensive enterohepatic circulation, i.e., are reabsorbed in the intestine, taken up again by the liver and re-secreted into bile. Therefore, hepatocytes excrete a mixture of primary bile acids derived from *de novo* synthesis and active ileal reabsorption as well as secondary bile acids which are formed and passively reabsorbed in the colon after bacterial dehydroxylation [13]. The liver expresses a range of specific uptake and export systems for biliary compounds in hepatocytes and cholangiocytes [2,8,14] which are summarized in Fig. 1.

Hepatobiliary transport systems are regulated at both transcriptional and post-transcriptional levels [8]. A major breakthrough has originated from the identification of ligand-activated NRs as major transcriptional

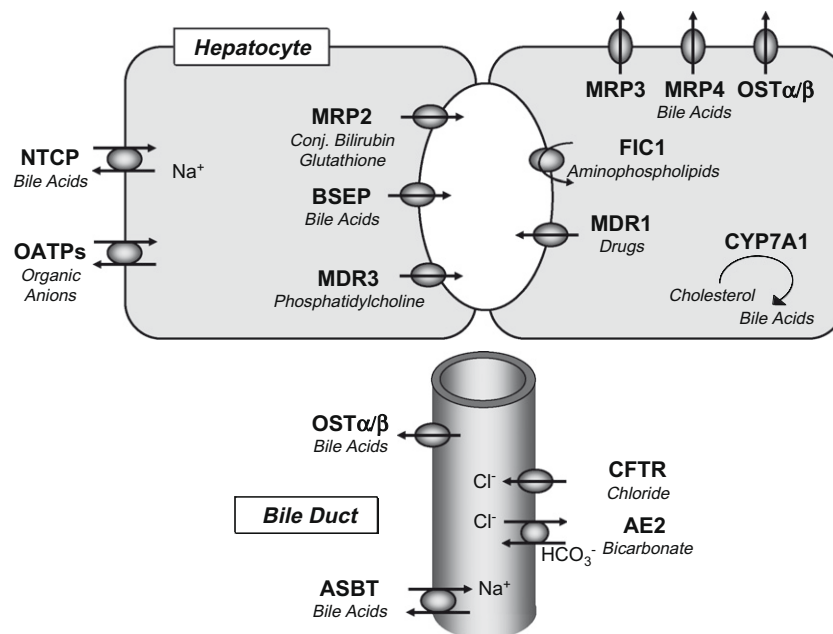


Fig. 1. Overview of hepatobiliary transporters. Hepatocellular bile acids are either derived from cholesterol by *de novo* synthesis via the key enzyme CYP7A1 replacing daily bile acid loss via stool (3–5%) or via hepatocellular uptake from the sinusoidal blood containing bile acids undergoing enterohepatic circulation. Hepatocellular bile acid uptake from the sinusoidal blood is mediated by a high-affinity Na⁺/taurocholate cotransporter (NTCP) and a family of multispecific organic anion transporters (OATPs). Canalicular excretion of bile constituents via specific ABC transporters represents the rate-limiting step of bile formation. The canalicular membrane contains a bile-salt export pump (BSEP) for monovalent bile acids; a conjugate export pump MRP2 mediates excretion of various organic anions such as bilirubin and divalent bile acids. The phospholipid export pump MDR3 flopps phosphatidylcholine from inner to outer leaflet, which forms mixed micelles together with bile acids and cholesterol. Cationic drugs are excreted by the multidrug export pump MDR1. Moreover, the canalicular membrane contains a P-type ATPase, FIC1, which is a putative aminophospholipid flippase. At the basolateral membrane additional bile acid export pumps, MRP3, MRP4 and the heterodimeric organic solute transporter OSTα/β are present as back up pumps for alternative sinusoidal bile acid export. Under normal conditions these transport systems are expressed at very low levels, but can be induced under cholestatic conditions or by therapeutic drugs. A chloride-bicarbonate anion exchanger isoform 2 (AE2), mediates biliary bicarbonate excretion in hepatocytes (not shown) and – to a greater extent – cholangiocytes. The cystic fibrosis transmembrane conductance regulator (CFTR) drives bicarbonate excretion by AE2 and is exclusively expressed in cholangiocytes. The biliary epithelium is also involved in the reabsorption of bile acids via an apical Na⁺-dependent bile-salt transporter ASBT and the basolateral counterpart OSTα/β. In addition, the bile duct epithelium expresses a range of channels and exchangers reviewed elsewhere [14].

regulators for positive feed forward and negative feedback pathways regulating bile formation under normal and pathological conditions [8,15]. Biliary constituents (e.g., bile acids), lipid products (e.g., oxysterols), hormones and xenobiotics (e.g., drugs) activate NRs as endogenous or exogenous ligands and thus coordinately regulate the expression of target genes that encode hepatobiliary transporters and phase I and II metabolism enzymes [2,16–18]. The best defined nuclear receptor for bile acids, farnesoid X receptor (FXR, NR1H4), is critically involved in the regulation of Na⁺-dependent (NTCP) and Na⁺-independent hepatocellular bile acid uptake (OATP1B1 and OATP1B3) [19,20], canalicular excretion of monovalent (BSEP) [21] and divalent bile acids (MRP2), conjugated bilirubin (MRP2) [22] and regulates the rate limiting step of bile acid production (CYP7A1) [23,24]. FXR either directly transactivates expression of genes (such as *BSEP*, *MRP2*, *MDR3*) or indirectly represses genes (e.g., *NTCP*, *OATP1B1* and *CYP7A1*) in a negative feedback loop via induction of the common gene silencer small heterodimer partner (SHP) [23–25] (Fig. 2). Collectively, activation of FXR reduces hepatocellular bile acid concentration and bile acid-induced toxicity and therefore represents a key molecular target in cholestasis. The classical xenobiotic receptors, pregnane X receptor (PXR, NR1I2), the constitutive androstane receptor (CAR, NR1I3) and the vitamin D receptor (VDR, NR1I1) are ligand-activated NRs which also sense cholephiles (e.g., bilirubin) in addition to bile acids. These NRs are mainly involved in regulation of detoxification enzymes and export pumps for biliary compounds (for recent reviews see [2,16,17]). The principal regulatory NR networks are summarized in Fig. 2. To add more complexity, transcriptional regulation by NRs also involves the binding and recruitment of co-activators and co-repressors [26]. Impaired co-regulator function have been described in metabolic diseases such as Von Gierke's disease [27] and play a critical role in the glucose and fatty acid metabolism of the liver [26]. Co-regulator alterations may also play a role in the pathogenesis of cholestasis and could represent potential targets for future therapeutic interventions [28]. For the specific example of FXR, there is a growing body of evidence that NR activity is also affected by chromatin remodelling by histone modification through acetylation (e.g., via steroid receptor coactivator-1, SRC-1, or p300 acetylase or methylation (e.g., via coactivator-associated arginine methyltransferase 1, CARM1 or protein arginine methyltransferase 1 PRMT1) [29]. In addition to transcriptional mechanisms, post-transcriptional events affecting mRNA processing, steady-state mRNA stability, translational efficacy and/or posttranslational changes such as transporter targeting and sorting, transporter redistribution, transporter protein degradation (e.g., via lysosomal or ubiquitin-proteasome pathway),

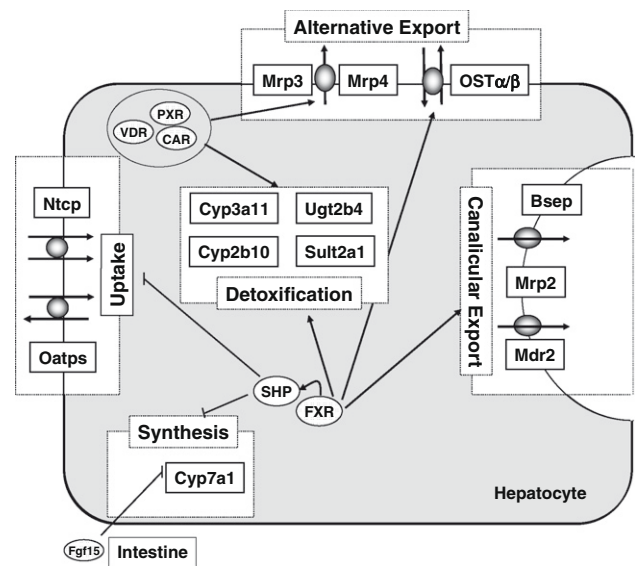


Fig. 2. Main principles of nuclear receptor-dependent regulation of hepatobiliary bile acid transporters and enzymes. Schematic representation of a rodent “model hepatocyte”. The principal transcriptional processes in humans are similar but less well explored. The central regulator of bile formation is bile acid activated Fxr, either as direct positive regulator for canalicular bile acid export via Bsep and Mrp2 as well as phospholipid export via Mdr2 or indirectly as repressor via the common transcriptional repressor Shp for basolateral Na⁺-dependent, Ntcp and Na⁺-independent, Oatps, bile acid uptake as well as bile acid synthesis via Cyp7a1. In addition, Fgf15, derived from ileal enterocytes, also strongly represses Cyp7a1 transcription and therefore represents an intestinal sensor for bile acid requirement. Except Ostα/β which is also regulated by Fxr, alternative export systems are independent of Fxr. Pxr and Vdr positively regulate Mrp3, while Car positively regulates both Mrp3 and Mrp4. Phase I (Cyp3a11 and Cyp2b10) and phase II (Sult2a1) metabolism is stimulated by Car and Pxr, but also Fxr is able to stimulate phase I detoxification via Cyp3a11 and phase II glucuronidation and sulfation via Ugt2b4 and Sult2a1.

direct protein modifications (e.g., (de-) phosphorylation, (de-) glycosylation), changes in membrane fluidity or cis-/trans-inhibition of transport systems by cholestatic agents (e.g., drugs) also modulate transporter expression and function [1,3,8,30].

3. Genetic defects and modifier genes in cholestasis

Hereditary defects of hepatobiliary ATP-binding cassette (ABC) transporters have been linked to a broad spectrum of hepatobiliary disorders ranging from progressive familial intrahepatic cholestasis (PFIC), benign recurrent intrahepatic cholestasis (BRIC) to intrahepatic cholestasis of pregnancy (ICP), drug-induced cholestasis, intrahepatic cholelithiasis and adult biliary cirrhosis (Fig. 3). Homozygous high impact variants cause progressive cholestatic syndromes in neonates and children [31,32], while lower impact variants may result in cholestatic syndromes which manifest later in juvenile or even adult life [32,33]. Moreover, heterozygous transport

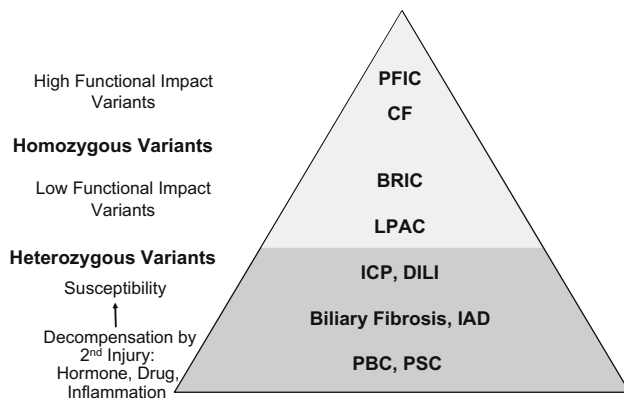


Fig. 3. Hereditary transporter defects: a continuous spectrum of liver disease. High impact variants of homozygous transporter mutations as tip of the pyramid can result in autosomal recessively inherited syndromes of progressive familial intrahepatic cholestasis (PFIC) (mutations in *FIC1*, *BSEP* or *MDR3* genes) and liver involvement in cystic fibrosis (CF) (mutations in *CFTR* gene) in early childhood. Low impact variants of homozygous mutations in *FIC1* or *BSEP* and *MDR3* cause benign recurrent intrahepatic cholestasis (BRIC) and low-phospholipid associated cholelithiasis (LPAC) in young adults. BRIC patients can progress to more aggressive disease indicating that PFIC and BRIC may belong to a continuous spectrum of pathophysiologically related conditions. Heterozygous *MDR3* and – to a lesser extent – *BSEP* mutations and polymorphisms may increase the susceptibility for acquired cholestatic injury such as intrahepatic cholestasis of pregnancy (ICP), drug induced liver injury (DILI), idiopathic adulthood ductopenia (IAD), biliary fibrosis or may play a role as modifier genes in more “classic” cholangiopathies (e.g., primary biliary cirrhosis, PBC; and primary sclerosing cholangitis, PSC). As such, a second hit/injury such as inflammation, drugs or hormones may lead to decompensation and disease development and/or determines disease progression.

defects may predispose to acquired cholestatic injury through decompensation of latent/mild defects under challenge with drugs, hormones and/or inflammatory mediators [34]. Polymorphisms in the promoter and coding regions of transporter genes in healthy individuals have been associated with reduced hepatic expression of these proteins [35–37] and, therefore, may also constitute a susceptibility factor for the development of acquired cholestasis. The role of genetic variations of transporter expression and function for the pathogenesis of cholangiopathies such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), which constitute the major disease burden of chronic cholestatic syndromes in adults, is less clear [34,38], although recent data suggest that such polymorphisms (e.g., *AE2*, *MDR3* and *PXR*) may determine disease progression and response to therapy.

3.1. *MDR3* (*ABCB4*)

MDR3 is a flippase, translocating phospholipids (mainly phosphatidylcholine) from the inner to the outer leaflet of the canalicular membrane for subsequent extraction by bile acids [39]. Phospholipids in bile are required for formation of mixed micelles (with bile acids

and cholesterol) to protect the bile duct epithelium from the detergent properties of bile acids. Absence of biliary phospholipids results in formation of a toxic bile damaging apical membranes of cholangiocytes and hepatocytes [40,41]. The classic manifestation is PFIC-3 characterized by high GGT levels, bile duct disease and progressive cholestasis in infants leading to end-stage liver disease requiring liver transplantation in 50% of patients. *MDR3* variants are also associated with syndromic cholesterol cholelithiasis known as low-phospholipid-associated cholelithiasis syndrome (LPAC) which is characterized by cholesterol gallstones recurring after cholecystectomy, mild chronic cholestasis due to intrahepatic stones and an increased incidence of intrahepatic cholestasis of pregnancy (ICP) [42,43].

MDR3 mutations may also play an important role in acquired cholestatic disorders. Several studies have identified heterozygous *MDR3* mutations in patients with ICP [40,44,45]. These patients are healthy under normal conditions but exposure to high levels of sex hormones in pregnancy may unmask latent/mild defects and result in cholestasis. Patients with ICP due to *MDR3* mutations typically have elevated gamma-glutamyl transpeptidase (GGT) levels [44] although GGT levels may also be normal [46,47]. Patients who experienced ICP may develop subsequent chronic liver disease [40,48,49] which so far has largely been attributed to the higher susceptibility for biliary complications resulting from cholesterol gallstone formation [46,50]; future studies will have to show whether this may rather be linked to a *MDR3* cholangiopathy (see below). *MDR3* gene polymorphisms and mutations have been associated with drug-induced cholestasis [51]: patients with *MDR3* defects may experience cholestasis when challenged with drugs transported by *MDR3* (e.g., verapamil, cyclosporin A and vinblastine) thus impairing phospholipid secretion [52].

MDR3 mutations should also be considered in adult patients with otherwise unexplained chronic cholestasis and biliary fibrosis, since a recent study [53] has identified *MDR3* mutations in one-third of such patients after exclusion of other more common causes such as PBC, sclerosing cholangitis and granulomatous hepatitis. The role of *MDR3* in the pathogenesis of classic PBC and PSC is still controversial. While common *MDR3* polymorphisms could not be detected in Caucasian PBC patients, a recent Japanese study reported an association of *MDR3* haplotypes and diplotypes with disease progression of PBC [54–56]. These discrepancies might in part explain regional variations in disease course and progression but require further investigations. Since mice lacking *Mdr2* (the rodent homologue of human *MDR3*) develop a cholangiopathy with macroscopic and microscopic features of (primary) sclerosing cholangitis in humans [57], a role for *MDR3* defects in the pathogenesis of large bile duct diseases (including

PSC) has been proposed. So far, single *MDR3* variations have not been associated with pathogenesis of PSC [54,58], which indeed would have been quite unexpected regarding the complex pathophysiology of this disease. However, variations in the *MDR3* gene may influence disease progression in PSC patients and also disease susceptibility in interaction with other transporter gene polymorphisms as demonstrated for the organic solute transporter (*OST α*) gene [59]. *MDR3* variants could be more relevant in specific subphenotypes of PSC (e.g., pediatric PSC, female patients with gallstones) [60].

Moreover, *MDR3* defects have been linked to non-anastomotic intrahepatic bile duct strictures (NAS) after liver transplantation. Bile cytotoxicity, in particular a low biliary phospholipids/bile salt ratio may be involved in the pathogenesis of NAS [61], since biliary bile acid excretion recovers more rapidly than phospholipid excretion after liver transplantation, leading to a more hydrophobic biliary bile acid composition [62]. These data suggest that the impact of cold ischemia/reperfusion injury on *MDR3* expression and function – in particular in genetically susceptible individuals with an *MDR3* genetic variation could contribute to NAS. This hypothesis is further supported by findings in heterozygous *Mdr2*^{+/-} mice, which secrete only 50% of the normal amount of phosphatidylcholine into bile and developed bile duct injury and cholestasis after liver transplantation in contrast to their wild-type littermates [63].

3.2. *BSEP (ABCB11)*

BSEP is the main canalicular bile salt export pump for monovalent bile acids such as glycine- or taurine-amidates of cholic acid (CA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) [8]. BSEP is the driving force of bile salt-dependent bile flow and impaired expression and/or function of BSEP causes significant cholestasis. Mutations in the *BSEP* gene result in PFIC-2 [64]. In contrast to PFIC-3, liver damage in PFIC-2 is restricted to hepatocytes since bile acids do not reach bile canaliculi and bile ducts explaining normal GGT levels in these patients. Accumulation of bile acids in hepatocytes causes giant cell hepatitis and progressive liver damage disease requiring liver transplantation at early age [32]. Some children may also develop hepatocellular carcinoma, which might be linked to the mutagenic potential of bile acids [65,66]. A milder variant of PFIC-2 is benign intrahepatic recurrent cholestasis (BRIC-2), which is also associated with a high risk for the development of gallstones [67]. The distinction between progressive and benign forms of cholestasis in the present nomenclature suggests that these are entirely separate syndromes. However, recent data indicate that many of the so-called BRIC patients can progress to more aggressive disease over time, indicating that PFIC

and BRIC rather belong to a continuous spectrum of pathophysiologically related conditions.

Similar to *MDR3*, *BSEP* variants may also play a role in the pathogenesis of ICP [44,68]. Heterozygosity for the common *BSEP* mutations accounts for about 1% of European ICP cases [69]. A polymorphism leading to an exchange of valine to alanine at position 444 of the BSEP protein (V444A) is a significant risk factor for ICP [68,69]. Interestingly, ICP was associated with heterozygosity for the V444A polymorphism, while all patients with contraceptive-induced cholestasis were homozygous for this polymorphism [68]. This indicates that lower estrogen levels in contraceptive-induced cholestasis compared to second or third trimester pregnancy require two low-function alleles to result in cholestasis [68]. Since sulphated progesterone metabolites are increased in ICP [70], inhibition of BSEP function through such metabolites might also contribute to ICP [71]. In contrast to *MDR3*-associated ICP, *BSEP* polymorphisms in ICP do not lead to elevation of GGT levels [68] which may help to phenotypically distinguish *MDR3* and *BSEP*-related forms of ICP (similar to PFIC). The differentiation could be of clinical relevance, since patients with *MDR3* mutations may later develop chronic liver disease [40,48,49]. Combined homozygous mutations of both *BSEP* and *MDR3* have been described in one ICP patient causing even more severe cholestasis [72].

BSEP mutations have also been linked to drug-induced liver injury [51]. The V444A polymorphism has been associated with a threefold increased risk to develop cholestatic drug side effects in response to treatment with different drugs (i.e., beta-lactam antibiotics, proton pump inhibitors, oral contraceptives and psychotropic drugs) [51]. A functional explanation for the increased risk of cholestasis may lie in decreased BSEP expression levels in patients carrying this polymorphism [73]. So far, no strong role of *BSEP* genetic variants for the pathogenesis of cholangiopathies such as PBC and PSC could be established [54,55].

3.3. *FIC1 (ATB8B1)*

FIC1 is considered to be an inward flippase for phosphatidylserine which may be crucial for maintaining cell membrane asymmetry [74,75]. FIC1 is expressed broadly in tissues throughout the body including the urinary bladder, intestine, pancreas and stomach and hepatocytes, where it is localized to the canalicular membrane. FIC1 deficiency causes severe cholestatic liver disease indicating an important function in bile secretion. Mutations in the *FIC1* gene cause PFIC-1 [76], also known as Byler disease, presenting in the neonatal period and characterized by elevated levels of serum bile acids, bilirubin and transaminases, low biliary bile acid concentrations and low levels of GGT.

The disease rapidly progresses to end-stage liver disease requiring liver transplantation at early age. Consistent with the wide extrahepatic expression of FIC1, liver transplantation only resolves cholestasis, but not the extrahepatic manifestations such as diarrhea, bile acid malabsorption, pancreatitis and nephrolithiasis [77].

The exact pathomechanism by which FIC1 deficiency leads to cholestasis is still under investigation. FIC1 deficiency is associated with reduced FXR expression and function which may result in disturbance of intestinal and hepatic bile acid transporter expression [78–80]. This hypothesis was further strengthened in an *in vitro* model where FIC1 overexpression led to enhanced phosphorylation and nuclear localization of FXR associated with activation of its target gene *BSEP*. In line with this hypothesis, Byler disease *FIC1* mutants did not activate BSEP, and BRIC mutants only partially activated BSEP expression [80]. This contrasts the findings of a recent study, where FXR activity remained after FIC1 knock-down [81]. Another possible explanation for cholestasis in FIC1 deficiency leading to decreased or even absent flippase activity is the loss of phospholipid asymmetry of the canalicular membrane. This in turn destabilizes the canalicular membrane rendering it less resistant towards hydrophobic bile acids [74,81]. Furthermore, the loss in phospholipid asymmetry may reduce bile acid transport via reduced BSEP activity thus contributing to cholestasis [74,81].

A milder form of PFIC-1 also caused by phenotypically less severe mutations in the *FIC1* gene is BRIC-1 (Summerskill syndrome). BRIC-1 is characterized by recurrent episodes of cholestasis not necessarily leading to liver cirrhosis [76], although recent reports suggest that BRIC-1 may also progress to a form that is indistinguishable from PFIC-1 [82]. One might speculate that in BRIC-1 patients residual activity of FIC1 may still be present, while in PFIC-1 patients this activity is completely lost [32]. Consistent with this concept, BRIC-1 and PFIC-1 may be considered the far ends of a continuous disease spectrum. Similar to *MDR3* and *BSEP*, mutations in the *FIC1* gene have also been linked to ICP [83,84]. Whether *FIC1* polymorphisms or mutations are associated with other cholestatic liver diseases remains to be determined.

3.4. MRP2 (*ABCC2*)

MRP2 mediates canalicular excretion of conjugated bilirubin and a broad range of organic anions (e.g., conjugates with glutathione, glucuronate and sulfate formed by phase II conjugation). MRP2-dependent excretion of reduced glutathione (GSH) critically determines bile acid-independent bile flow [85]. In addition, MRP2 transports divalent bile acids with two negative charges such as sulfated tauro- or glycolithocholate.

Mutations in the *MRP2* gene are found in patients with Dubin–Johnson syndrome resulting in jaundice while typical features of cholestasis such as elevated serum bile acid levels and pruritus are absent [86]. The only acquired cholestatic liver disease associated with *MRP2* mutations identified so far, is ICP [87]. Secondary alterations of this transporter (repression of transcription, posttranslational changes or direct inhibition of MRP2 function; see below) may play a more important role in the pathogenesis of cholestasis than hereditary defects.

3.5. AE2 (*SLC4A2*)

Anion exchanger AE2 mediates $\text{Cl}^-/\text{HCO}_3^-$ exchange thus regulating intracellular pH and biliary HCO_3^- excretion by hepatocytes and cholangiocytes. Expression of AE2 is reduced in patients with PBC and may contribute to reduced bile flow and cholestasis [88–90]. Moreover, AE2 expression is also reduced in salivary and lacrimal glands of PBC patients thus contributing to Sjögren's syndrome, which is frequently associated with PBC. Ursodeoxycholic acid (UDCA) treatment improves AE2 expression [89] and function [90]. UDCA and glucocorticoids synergistically stimulate AE2 promoter activity via interactions with hepatocyte nuclear factor 1 (HNF1) and the glucocorticoid receptor (GR, NR3C1). Conversely, progression of PBC despite UDCA therapy has been linked to allelic variations in the *AE2* gene [55]. The molecular link between AE2 and PBC is further supported by the findings in Ae2 knockout mice [91] which develop biochemical, serological, immunological and histopathological features of PBC including development of animitochondrial antibodies. Ae2 deficiency not only leads to disturbances in intracellular pH homeostasis in cholangiocytes but also in immunocytes which may explain the immunological and functional changes seen in PBC patients [91,92].

3.6. CFTR (*ABCC7*)

The cystic fibrosis transmembrane regulator (CFTR) is a cAMP-dependent chloride channel. Mutations in the *CFTR* gene cause cystic fibrosis [93]. Impaired bicarbonate and fluid secretion in CF may result in inspissated bile, which can be toxic to the bile duct epithelium. Hepatobiliary complications from CF occur in up to 25% of patients ranging from prolonged neonatal cholestasis, hepatic steatosis and focal nodular cirrhosis to multilobular cirrhosis and biliary tract complications such as stones and sclerosing cholangitis. Four percentage to 18% of adult CF patients show cholangiographic features of sclerosing cholangitis, sometimes even in association with inflammatory bowel disease [94]. Vice versa, induction of colitis in *Cftr* knockout mice causes bile duct injury [95] making

CFTR an interesting candidate gene for PSC. Interestingly, an increased prevalence of *CFTR* variants was observed in a subset of PSC patients with inflammatory bowel disease [96]. The identified *CFTR* mutations were also associated with a reduced chloride secretory response [96]. Another study observed a high prevalence of *CFTR* mutations and decreased *CFTR* function in childhood PSC [97]. However, several other studies could not confirm an association of disease-causing *CFTR* mutations with PSC [98,99] and a recent study by Henckaerts et al. even identified specific dysfunctional *CFTR*-variants that might protect against the development of PSC [99]. Thus, the underlying mechanisms still remain elusive and whether *CFTR* is indeed a modifier gene for development of PSC needs to be determined in larger numbers of patients.

3.7. Other genetic variants associated with cholestasis

In addition to genetic defects in transporter genes, variations in tight junction genes can also cause cholestasis. Missense mutations in *tight junction protein 2* have been identified in patients with familial hypercholelanemia, an oligogenic disease requiring a functional alteration in a second gene (i.e., *bile acid amino acid transferase*) for full phenotypic manifestation [100]. Furthermore, mutations of *claudin-1*, which forms the backbone of tight junctions with occludin and junctional adhesion molecules causes neonatal sclerosing cholangitis associated with ichthyosis [101]. Mutations in *JAG-1*, which is involved in notch signalling required for bile duct development, have been identified in patients with Alagille syndrome, an autosomal dominant disorder, which results in cholestasis due to bile duct paucity [102]. Inborn errors of bile acid synthesis are well established causes of neonatal cholestasis (for review see [103]). Genetic defects in nuclear receptors regulating gene expression of hepatobiliary transporters and enzymes may also be involved in cholestatic liver disease or potential response to therapy. The nuclear receptor FXR has been linked to ICP [104] and gallstone disease [105]. In addition, functional *PXR* gene variants appear to modify disease course in PSC and weakly in PBC [55,106].

4. Acquired defects and adaptation to cholestasis

In general, the acquired changes in transporter expression in human cholestatic liver diseases (alcoholic hepatitis, PBC, PSC, malignant obstruction) are consistent with concepts derived from the findings in experimental animal models of cholestasis [2,17,107]. These models include common bile duct ligation (CBDL, model for biliary duct obstruction), endotoxin challenge (sepsis/inflammation-induced cholestasis) or administra-

tion of ethinylestradiol (cholestasis of pregnancy) [108] and have permitted important mechanistic insights into cholestasis. Bile acid feeding is commonly used to investigate direct effects of bile acids without (peri-)operative stress or induction of pro-inflammatory cytokines as observed during CBDL [109].

While some of the acquired (e.g., cytokine-mediated) alterations contribute to cholestasis [2], other (e.g., bile acid-induced) changes may represent compensatory ('anti-cholestatic') defense mechanisms which provide alternative excretory routes for accumulating cholephiles in cholestasis. Rising intrahepatic and systemic bile acid levels under cholestatic conditions orchestrate an adaptive response, which is mainly coordinated by a complex interplay of bile acid and bilirubin-activated nuclear receptors (mainly FXR, PXR and CAR), in an attempt to counteract cholestatic liver injury. As a result of this transcriptional program, basolateral bile acid uptake systems are markedly reduced (e.g., NTCP, OATPs), while basolateral bile acid export pumps are increased simultaneously (e.g., MRP3, MRP4 and OST α/β), which is considered to be a major hepatocellular defense mechanism counteracting intracellular bile acid toxicity [16,17,108] (Fig. 4). In addition, bile acid hydroxylation and conjugation via phase I and phase II enzyme reactions reduces toxicity and increases water solubility for subsequent alternative elimination of cholephiles via the urine [3,16,108,110]. These adaptive modulations in response to cholestasis are not only restricted to the liver but also occur in intestine, kidney and bile duct epithelia [16,17,108] (Fig. 4). Intestinal and renal bile acid uptake and export transporter systems can adapt to local bile acid concentrations and thus are able to increase fecal and/or urinary bile acid elimination. In obstructed bile ducts, bile acid reuptake and delivery to the liver for subsequent detoxification is increased [16,17,108]. Unfortunately this armamentarium of adaptive responses is apparently too weak to fully prevent cholestatic injury. Part of this may be due to the fact, that cholestatic/inflammatory injury results in repression of nuclear receptor expression and function [111–113]. However, these responses might be targeted/enhanced therapeutically by nuclear receptor activating drugs.

4.1. Obstructive cholestasis

Biliary obstruction in rodents initiates marked changes in transporter expression. As an important protective regulatory step, the basolateral Na⁺-dependent bile acid uptake system, Ntcp, which extracts the great bulk of bile acids returning to the liver is downregulated to prevent bile acid uptake into hepatocytes [114]. Accumulation of bile acids activates Fxr and subsequently induces Shp resulting in repression of Ntcp [25,115] (Fig. 2). The importance of this mechanism is underlined

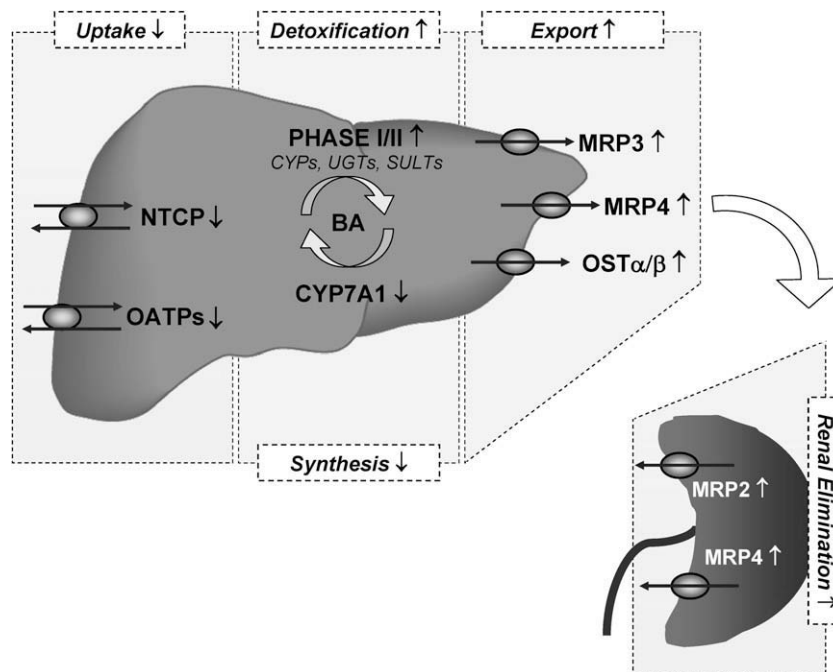


Fig. 4. Overview of adaptive response in cholestasis. Cholestasis results in intrahepatic accumulation of bile acids. To limit toxic hepatocellular bile acid burden further uptake of bile acids is restricted by downregulation of NTCP and OATPs. Intrahepatic *de-novo* synthesis of bile acids is downregulated via CYP7A1, mediating the rate limiting step in bile acid biosynthesis. Moreover, intrahepatic bile acids can be detoxified via phase I hydroxylation and phase II conjugation reactions mediated via cytochrome p450 enzymes, glucuronidases and sulfatases. These modifications render intrahepatic bile acids more hydrophilic and facilitate their elimination. Export of bile acids in cholestasis is enhanced by upregulation of basolateral adaptive overflow transporters, such as MRP3, MRP4 and OST α/β , which are normally expressed only at low levels under physiological conditions. Hydrophilic bile acids can then be eliminated via the kidney either via glomerular filtration or potentially also via additional active ATP-transporter mediated tubular secretion. In addition, reduced intestinal bile acid uptake via reduced expression of ASBT contributes to reduced systemic bile acid accumulation via enhancing fecal excretion of bile acids (not shown).

by unchanged Ntcp levels in *Fxr* knockout animals after CBDL [116]. In contrast, CBDL in Shp knockout mice results in downregulation of Ntcp, suggesting that Shp-independent mechanisms might also be involved [117]. Such mechanisms may involve bile acid-dependent suppression of the Hnf4 α /Hnf1 α pathway as a key pathway of Ntcp gene regulation [2,118]. In contrast to bile acid uptake, expression of the major canalicular bile acid exporter Bsep, is largely maintained during biliary obstruction and may limit the extent of liver injury resulting from bile acid retention particularly when cholestasis is prolonged [119]. However, enhancing bile flow in the presence of complete biliary obstruction may aggravate cholestatic liver injury through development of bile infarcts [57]. Indeed, *Fxr* knockout animals, which have lower bile flow and biliary pressure, develop fewer bile infarcts as typical lesion in obstructive cholestasis [120]. These results may imply some precautions for the potential use of bile flow stimulating drugs in cholestatic liver disease, especially those with a significant obstructive component (e.g., PSC) [120,121].

The mechanism of alternative basolateral Mrp3 and Mrp4 induction in obstructive cholestasis has not yet been fully elucidated. NRs such as Pxr and Car, which can be activated by accumulated cholephiles such as bile

acids or bilirubin, or NF-E2-related factor (Nrf2), which is a common regulator of basolateral Mrp expression may be involved [122,123] (Fig. 2). Pxr and Car agonists induce these basolateral overflow system Mrp3 and Mrp4 together with phase I and phase II detoxification enzymes, thereby lowering biochemical markers of cholestasis [124]. These effects are not seen in the respective knockout animals under cholestatic conditions [121]. However, the overall importance of single basolateral transporters as adaptive overflow systems should not be overestimated, since Mrp3 and Mrp4 knockout animals do not show an overtly aggravated cholestatic phenotype in CBDL [125,126] and so far no significant human mutations or polymorphisms in *MRP3* and *MRP4* have been reported. The importance of upregulation of heteromeric OST/Ost α/β [127], which is a FXR/Fxr dependent basolateral bile acid overflow system, for obstructive cholestasis still needs to be determined, but underlines again the central role of FXR/Fxr in regulating bile acid shuttling in and out of hepatocytes (Fig. 2).

Cyp7a1, the rate limiting enzyme in bile acid biosynthesis is paradoxically induced in obstructive cholestasis and may further contribute to bile acid accumulation via continuous endogenous bile acid production [128,129].

Physiologically, a sophisticated gut to liver signaling cascade critically regulates the demand of bile acids from an “intestinal perspective” [130]. Fibroblast growth factor 15 (Fgf15), a hormone-like factor, is induced by bile acid binding to Fxr in the terminal ileum and signals from the gut to the liver, where it represses bile acid biosynthesis in concert with Shp via downregulation of Cyp7a1 [131] (Fig. 2). In obstructive cholestasis, when enterocytes face only small amounts of bile acids, ileal Fgf15 is low and hepatic Cyp7a1 levels inappropriately high [131]. Therapeutic activation of ileal Fxr is therefore thought to reduce bile acid burden under obstructive cholestatic conditions and might significantly contribute to the beneficial effects of GW4064, an Fxr agonist with a poor absorptive rate, in CBDL rats [132] (Fig. 4). The role of human FGF19 signalling (the human ortholog of murine Fgf15) in human obstructive cholestasis and its potential effects on bile acid synthesis is, however, less clear and still needs to be defined [133,134].

In human cholestatic liver disease, most changes in hepatobiliary transporters and enzymes may be interpreted as a consequence of elevated bile acid levels and represent adaptive mechanisms similar to cholestatic animal models. In early stage PBC and PSC, when bilirubin and bile acid levels are still within the normal range, hepatobiliary transporter expression is not changed. Late stage cholangiopathies or malignant extrahepatic cholestasis, however, results in repression of basolateral uptake systems and induction of basolateral overflow pumps such as MRP3, MRP4 and OST α/β [111,135,136].

The reduction of intestinal bile acids in obstructive cholestasis results in disruption of intestinal barrier integrity and mucosal defense leading to endotoxemia and potential worsening and perpetuation of the cholestatic condition. Bile acids inhibit bacterial growth *in vitro* and bacterial overgrowth and translocation *in vivo* [137,138]. Conversely, absence of bile leads to bacterial overgrowth, mucosal injury, bacterial translocation and endotoxemia in the small intestine [138,139]. Again, the bile acid receptor Fxr, has been linked as a central integrator to intestinal mucosal defense [139]. Fxr agonists are able to block bacterial overgrowth, translocation, mucosal injury and neutrophil extravasation in common bile duct ligated mice, while Fxr knockout mice have higher intestinal bacterial counts, higher rates of bacterial translocation and mucosal injury [139]. Thus, a picture is emerging where under physiological conditions bile acids inhibit bacterial overgrowth in the proximal small intestine directly and in the distal small intestine indirectly by their signalling properties via Fxr. Again, this concept would favour therapeutic FXR activation in the gut in cholestatic conditions.

4.2. Inflammation-induced cholestasis

Cholestasis frequently occurs as a complication in patients with sepsis, extrahepatic bacterial infections and alcoholic hepatitis [140,141]. Endotoxin (lipopolysaccharide, LPS) and LPS-induced pro-inflammatory cytokines such as tumor necrosis factor (TNF) α , interleukin-1 (IL-1) β and interleukin-6 (IL-6) are secreted mainly by macrophages and Kupffer cells [142,143], but hepatocytes *per se* can be a source of proinflammatory cytokines [144]. Pro-inflammatory cytokines are potent inhibitors of hepatobiliary transport and NR expression and function [2,145,146]. The transport of organic anions at both the sinusoidal and canalicular membrane of hepatocytes is reduced and both bile acid-dependent and bile acid-independent fractions of bile flow are affected [147,148].

LPS leads to a rapid and marked down-regulation of Ntcp [149,150] via decreased binding activity of the nuclear transcription factors Hnf1 α and Rxr α :Rar α to the (rat) Ntcp gene promoter. In contrast to CBDL, LPS-induced cytokines reduce Ntcp expression without induction of Shp [116,151]. Reduced canalicular secretion of bile acids and other organic anions during endotoxemia is caused by a concomitant reduction of the canalicular export pumps Bsep and Mrp2 [119,152]. Transcriptional control of Bsep and Mrp2 is mediated by a group of nuclear hormone receptors including Fxr, Rar, Pxr and Car (Fig. 2) [21,22,153,154]. Phosphorylation and nuclear export of the common NR heterodimer partner Rxr α represents one component of the uniformly decreased transporter gene expression during LPS-induced cholestasis [155,156]. Furthermore, LPS-induced down-regulation of Bsep mRNA may be mediated by suppression of Fxr [21,157]. In addition, decreased activity of Pxr and Car may be important for Mrp2 repression [112,157,158] (Fig. 2). In the regulation of the human transporter genes under inflammatory conditions posttranscriptional mechanisms play a more prominent role. In LPS-treated human liver slices BSEP and MRP2 mRNA levels are unaltered whereas both proteins are virtually absent under these conditions [159]. Similarly, reductions in canalicular BSEP and MRP2 staining have been observed in liver biopsies from patients with inflammation-induced cholestasis [160]. Transporter retrieval from the canalicular membrane has been described as a potential posttranscriptional mechanism of early and reversible regulation of Mrp2 during endotoxemia [161]. In contrast to canalicular transporter regulation, much less is known about the regulation of the basolateral overflow systems during inflammatory cholestasis (for review [162]).

The understanding of inflammation-induced cholestasis is further expanded by findings demonstrating interaction of nuclear receptors with the inflammatory response cascade. Inflammation-induced activation of

nuclear factor- κ B (NF- κ B), which is a key transcriptional regulator of classical pro-inflammatory target genes such as TNF α , antagonizes Fxr activity and Fxr target gene expression and livers from *Fxr* knockout mice are more susceptible to inflammatory (e.g., LPS-induced) stress than wildtype animals [145]. Reciprocally, Fxr activation inhibits hepatocellular NF- κ B activation [145]. In cells which do not express FXR/Fxr (e.g., Kupffer cells/macrophages) anti-inflammatory and immunosuppressive properties of bile acids may be mediated via the membrane-bound G-protein-coupled bile acid receptor TGR5/Tgr5 [163,164].

4.3. Drug-induced cholestasis, steroid-induced cholestasis

Drugs can cause cholestasis by inhibiting hepatocellular transporter expression and function and, in rare cases, by inducing a vanishing bile duct syndrome, which can progress to biliary cirrhosis [36]. Many cases of drug-induced cholestasis result from a functional inhibition of transport proteins by the drug itself or its metabolites. Drugs can directly cis-inhibit ATP-dependent bile acid export via Bsep in a competitive manner (e.g., cyclosporine, rifampin, bosentan, troglitazone and glibenclamide) [165–167]. In addition, steroid hormones such as estrogen and progesterone metabolites are able to indirectly trans-inhibit Bsep function after their secretion into bile via Mrp2 [165]. Inhibition of MRP2 by drugs, such as the antibiotic fusidate, may also contribute to cholestasis and jaundice [168]. Phospholipid secretion via MDR3 might be impaired by verapamil, cyclosporin A, and vinblastine, since these compounds are transported by MDR3 *in vitro* [52]. Last but not least, genetic variations in transporter genes can predispose to drug- and steroid-induced cholestasis (see above). The detailed molecular mechanisms of drug- and steroid induced cholestasis have recently been reviewed elsewhere [169]. However, it has to be kept in mind that many adverse hepatic drug reactions are accompanied by an inflammatory element, and therefore may more resemble the features of inflammation-associated cholestasis.

5. Therapeutic strategies and outlook

So far, the only approved drug for treatment of cholestatic disorders is ursodeoxycholic acid (UDCA), which is a hydrophilic bile acid and normally accounts for only small parts of the human bile acid pool [110]. Proposed anti-cholestatic mechanisms of UDCA action include stimulation of canalicular efflux pumps (i.e., MRP2, BSEP) at a transcriptional and posttranscriptional level for “orthograde” biliary excretion and stimulation of basolateral export pumps (i.e., MRP3, MRP4) for adaptive alternative excretion of bile acids back from

hepatocytes into the systemic circulation [111,170–173]. In addition, UDCA may also have antiapoptotic and antifibrotic properties contributing at least to some of the beneficial effects [110]. However, in prototypic cholestatic liver diseases such as PBC, up to one to two third of patients only incompletely respond to UDCA and remain at continued increased risk for disease progression to cirrhosis and potential need for liver transplantation [174,175]. UDCA has no proven benefit in PSC [176–178]. Therefore, additional therapeutic strategies for chronic cholangiopathies such as PBC and PSC are required.

Currently, a promising novel treatment option for cholestasis are FXR agonists (e.g., 6-ethylchenodeoxycholic acid) and phase II studies have already been initiated for PBC. At least in rats, FXR/Fxr agonists were shown to improve intrahepatic and extrahepatic cholestasis [132]. Therapeutic FXR ligands could overcome the reduction of bile flow in cholestasis via stimulation of BSEP (increasing bile acid-dependent bile flow) and MRP2 (increasing bile acid-independent bile flow) [22,179]. FXR agonists may also support some adaptive reactions of the cholestatic hepatocyte to limit the hepatocellular bile acid burden, such as down-regulation of bile acid import and induction of basolateral overflow systems as well as repression of endogenous bile acid synthesis [2,16,108]. In addition, stimulation of the canalicular phospholipid flippase MDR3 via FXR is predicted to render bile composition less aggressive [180,181].

NorUDCA, is a side chain shortened UDCA derivate and promising candidate for sclerosing cholangitis [182]. Its relative resistance to amidation results in enhanced cholehepatic shunting from the bile duct lumen back to the hepatocytes, thereby inducing a bicarbonate rich and potentially less toxic bile flow [183]. In addition, *norUDCA* is enriched in the hepatocytes by this mechanism and markedly induces phase I and phase II detoxification enzymes and alternative basolateral overflow systems via yet unidentified transcriptional mechanisms [182]. Similar to UDCA, no definite nuclear receptor for the action of *norUDCA* has been defined. In contrast to UDCA, *norUDCA* heals sclerosing cholangitis in a mouse model resembling PSC [182]. The beneficial impact of *norUDCA* on bile acid metabolism and transport are accompanied by anti-fibrotic and anti-inflammatory effects [182]. Since stimulation of bicarbonate-rich choleresis by *norUDCA* is mostly independent from Cfr in mice, this drug may also be an interesting treatment option for CFTR-associated cholangiopathies [184].

A range of additional nuclear (e.g., PXR, CAR and PPAR α) and bile acid (e.g., TGR5) receptor agonists, which impact on detoxification and alternative export mechanisms, bile composition, fibrosis and inflammatory responses are currently under investigation with

promising results in animal models of cholestasis. Some drugs such as rifampicin and phenobarbital have been used in the pre-UDCA area for the treatment of cholestatic pruritus and jaundice and later turned out to be activators of PXR and CAR, respectively [185,186]. Also agonists of peroxisome proliferator-activated receptor alpha (PPAR α , NR1C1) are promising therapeutic approaches in human cholestatic liver disease since fibrates showed beneficial effects on biochemical parameters of cholestasis and/or transaminases in PBC patients with suboptimal response to UDCA [187–189]. A proposed mode of action could be stimulation of MDR3/Mdr2 by PPAR α agonists [190,191]. The future could bring us ‘cocktails’ of gene-selective agonists in order to specifically target subsets of genes and separate desired from unwanted effects. Another future level of intervention could be co-activators/co-repressors and histone modifying enzymes whose expression may also be altered in cholestatic and metabolic liver diseases [26].

In summary, the molecular mechanisms leading to cholestasis and protecting from cholestasis are complex. While some of these hereditary or acquired changes result in reduced transporter function and cholestasis, most of the observed changes are consequences rather than cause of cholestasis. Both primary and secondary alterations and their underlying regulatory mechanism are worthwhile pharmacological targets for the future treatment of cholestasis.

Acknowledgements

This work was supported by grants P18613-B05, P19118-B05 and F3008-B05 (to M.T.) from the Austrian Science Foundation and a GENAU project grant from the Austrian Ministry of Science.

References

- Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 1998;339:1217–1227.
- Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. *Biochim Biophys Acta* 2007;1773:283–308.
- Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology* 2004;126:322–342.
- Lazaridis KN, Strazzabosco M, LaRusso NF. The cholangiopathies: disorders of biliary epithelia. *Gastroenterology* 2004;127:1565–1577.
- Fava G, Glaser S, Francis H, Alpini G. The immunophysiology of biliary epithelium. *Semin Liver Dis* 2005;25:251–264.
- O’Mahony CA, Vierling JM. Etiopathogenesis of primary sclerosing cholangitis. *Semin Liver Dis* 2006;26:3–21.
- Gershwin ME, Mackay IR. The causes of primary biliary cirrhosis: convenient and inconvenient truths. *Hepatology* 2008;47:737–745.
- Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* 2003;83:633–671.
- Hofmann AF. The continuing importance of bile acids in liver and intestinal disease. *Arch Intern Med* 1999;159:2647–2658.
- Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. *Hepatology* 1991;14:551–566.
- Baiocchi L, LeSage G, Glaser S, Alpini G. Regulation of cholangiocyte bile secretion. *J Hepatol* 1999;31:179–191.
- Marzoni M, Glaser SS, Francis H, Phinizz JL, LeSage G, Alpini G. Functional heterogeneity of cholangiocytes. *Semin Liver Dis* 2002;22:227–240.
- Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. *Front Biosci* 2009;14:2584–2598.
- Strazzabosco M, Fabris L, Spirli C. Pathophysiology of cholangiopathies. *J Clin Gastroenterol* 2005;39:S90–S102.
- Houten SM, Auwerx J. The enterohepatic nuclear receptors are major regulators of the enterohepatic circulation of bile salts. *Ann Med* 2004;36:482–491.
- Zollner G, Marschall HU, Wagner M, Trauner M. Role of nuclear receptors in the adaptive response to bile acids and cholestasis: pathogenetic and therapeutic considerations. *Mol Pharm* 2006;3:231–251.
- Wagner M, Trauner M. Transcriptional regulation of hepatobiliary transport systems in health and disease: implications for a rationale approach to the treatment of intrahepatic cholestasis. *Ann Hepatol* 2005;4:77–99.
- Boyer JL. Nuclear receptor ligands: rational and effective therapy for chronic cholestatic liver disease? *Gastroenterology* 2005;129:735–740.
- Jung D, Podvinec M, Meyer UA, Mangelsdorf DJ, Fried M, Meier PJ, et al. Human organic anion transporting polypeptide 8 promoter is transactivated by the farnesoid X receptor/bile acid receptor. *Gastroenterology* 2002;122:1954–1966.
- Kullak-Ublick GA, Ismail MG, Stieger B, Landmann L, Huber R, Pizzagalli F, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* 2001;120:525–533.
- Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* 2001;276:28857–28865.
- Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J Biol Chem* 2002;277:2908–2915.
- Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 2000;6:517–526.
- Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 2000;6:507–515.
- Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, et al. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology* 2001;121:140–147.
- Han SJ, Lonard DM, O’Malley BW. Multi-modulation of nuclear receptor coactivators through posttranslational modifications. *Trends Endocrinol Metab* 2009;20:8–15.
- Chopra AR, Louet JF, Saha P, An J, Demayo F, Xu J, et al. Absence of the SRC-2 coactivator results in a glycogenopathy resembling Von Gierke’s disease. *Science* 2008;322:1395–1399.
- Walsh MJ, Ananthanarayanan M, Li S, Balasubramanian N, Suchy FJ. The steroid receptor co-activator 2 (SRC2) regulates the expression of the FXR target genes, bile salt export pump (ABCB11) and small heterodimer partner (SHP). *Hepatology* 2007;46:314A.

- [29] Suchy FJ, Ananthanarayanan M. Bile salt excretory pump: biology and pathobiology. *J Pediatr Gastroenterol Nutr* 2006;43 (Suppl. 1):S10–S16.
- [30] Anwer MS. Cellular regulation of hepatic bile acid transport in health and cholestasis. *Hepatology* 2004;39:581–590.
- [31] Jansen PL, Muller M, Sturm E. Genes and cholestasis. *Hepatology* 2001;34:1067–1074.
- [32] Oude Elferink RP, Paulusma CC, Groen AK. Hepatocanalicular transport defects: pathophysiologic mechanisms of rare diseases. *Gastroenterology* 2006;130:908–925.
- [33] Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. *Clin Liver Dis* 2000;4:753–763.
- [34] Pauli-Magnus C, Stieger B, Meier Y, Kullak-Ublick GA, Meier PJ. Enterohepatic transport of bile salts and genetics of cholestasis. *J Hepatol* 2005;43:342–357.
- [35] Saito S, Iida A, Sekine A, Miura Y, Ogawa C, Kawauchi S, et al. Three hundred twenty-six genetic variations in genes encoding nine members of ATP-binding cassette, subfamily B (ABCB/MDR/TAP), in the Japanese population. *J Hum Genet* 2002;47:38–50.
- [36] Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. *Hepatology* 2006;44:778–787.
- [37] Lang T, Haberl M, Jung D, Drescher A, Schlagenhauser R, Keil A, et al. Genetic variability, haplotype structures, and ethnic diversity of hepatic transporters MDR3 (ABCB4) and bile salt export pump (ABCB11). *Drug Metab Dispos* 2006;34:1582–1599.
- [38] Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, et al. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2004;127:261–274.
- [39] Oude Elferink RP, Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein). *Pflugers Arch* 2007;453:601–610.
- [40] Jacquemin E, de Vree JM, Cresteil D, Sokal EM, Sturm E, Dumont M, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology* 2001;120:1448–1458.
- [41] de Vree JM, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. *Proc Natl Acad Sci USA* 1998;95:282–287.
- [42] Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. *Gastroenterology* 2003;125:452–459.
- [43] Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. *Orphanet J Rare Dis* 2007;2:29.
- [44] Pauli-Magnus C, Lang T, Meier Y, Zodan-Marin T, Jung D, Breyman C, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics* 2004;14:91–102.
- [45] Wasmuth HE, Glantz A, Keppeler H, Simon E, Bartz C, Rath W, et al. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. *Gut* 2007;56:265–270.
- [46] Schneider G, Paus TC, Kullak-Ublick GA, Meier PJ, Wienker TF, Lang T, et al. Linkage between a new splicing site mutation in the MDR3 alias ABCB4 gene and intrahepatic cholestasis of pregnancy. *Hepatology* 2007;45:150–158.
- [47] Floreani A, Carderi I, Paternoster D, Soardo G, Azzaroli F, Esposito W, et al. Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. *Aliment Pharmacol Ther* 2006;23:1649–1653.
- [48] Jacquemin E. Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: one gene for three diseases. *Semin Liver Dis* 2001;21:551–562.
- [49] Lucena JF, Herrero JI, Quiroga J, Sangro B, Garcia-Foncillas J, Zabalegui N, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. *Gastroenterology* 2003;124:1037–1042.
- [50] Lammert F, Marschall HU, Glantz A, Matern S. Intrahepatic cholestasis of pregnancy: molecular pathogenesis, diagnosis and management. *J Hepatol* 2000;33:1012–1021.
- [51] Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. *Pharmacogenet Genomics* 2007;17:47–60.
- [52] Smith AJ, van Helvoort A, van Meer G, Szabo K, Welker E, Szakacs G, et al. MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. *J Biol Chem* 2000;275:23530–23539.
- [53] Ziol M, Barbu V, Rosmorduc O, Frassati-Biaggi A, Barget N, Hermelin B, et al. ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. *Gastroenterology* 2008;135:131–141.
- [54] Pauli-Magnus C, Kerb R, Fattinger K, Lang T, Anwald B, Kullak-Ublick GA, et al. BSEP and MDR3 haplotype structure in healthy Caucasians, primary biliary cirrhosis and primary sclerosing cholangitis. *Hepatology* 2004;39:779–791.
- [55] Poupon R, Ping C, Chretien Y, Corpechot C, Chazouilleres O, Simon T, et al. Genetic factors of susceptibility and of severity in primary biliary cirrhosis. *J Hepatol* 2008;49:1038–1045.
- [56] Ohishi Y, Nakamura M, Iio N, Higa S, Inayoshi M, Aiba Y, et al. Single-nucleotide polymorphism analysis of the multidrug resistance protein 3 gene for the detection of clinical progression in Japanese patients with primary biliary cirrhosis. *Hepatology* 2008;48:853–862.
- [57] Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Weiglein AH, Lammert F, et al. Ursodeoxycholic acid aggravates bile infarcts in bile duct-ligated and Mdr2 knockout mice via disruption of cholangioles. *Gastroenterology* 2002;123:1238–1251.
- [58] Rosmorduc O, Hermelin B, Boelle PY, Poupon RE, Poupon R, Chazouilleres O. ABCB4 gene mutations and primary sclerosing cholangitis. *Gastroenterology* 2004;126:1220–1222, author reply 1222–1223.
- [59] Melum E, Boberg KM, Franke A, Bergquist A, Hampe J, Karlsen TH. Variations in the MDR3 gene influences disease progression in PSC patients and disease susceptibility in epistatic interaction with a polymorphism in the OST-alpha gene. *Hepatology* 2007;46:265A.
- [60] Trauner M, Fickert P, Wagner M. MDR3 (ABCB4) defects: a paradigm for the genetics of adult cholestatic syndromes. *Semin Liver Dis* 2007;27:77–98.
- [61] Buis CI, Geuken E, Visser DS, Kuipers F, Haagsma EB, Verkade HJ, et al. Altered bile composition after liver transplantation is associated with the development of nonanastomotic biliary strictures. *J Hepatol* 2009;50:69–79.
- [62] Geuken E, Visser D, Kuipers F, Blokzijl H, Leuvenink HG, de Jong KP, et al. Rapid increase of bile salt secretion is associated with bile duct injury after human liver transplantation. *J Hepatol* 2004;41:1017–1025.
- [63] Hoekstra H, Porte RJ, Tian Y, Jochum W, Stieger B, Moritz W, et al. Bile salt toxicity aggravates cold ischemic injury of bile ducts after liver transplantation in Mdr2+/- mice. *Hepatology* 2006;43:1022–1031.
- [64] Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, et al. A gene encoding a liver-specific ABC transporter

- is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* 1998;20:233–238.
- [65] Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. *Hepatology* 2006;44:478–486.
- [66] Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat Res* 2005;589:47–65.
- [67] van Mil SW, van der Woerd WL, van der Brugge G, Sturm E, Jansen PL, Bull LN, et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. *Gastroenterology* 2004;127:379–384.
- [68] Meier Y, Zodan T, Lang C, Zimmermann R, Kullak-Ublick GA, Meier PJ, et al. Increased susceptibility for intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis in carriers of the 1331T>C polymorphism in the bile salt export pump. *World J Gastroenterol* 2008;14:38–45.
- [69] Dixon PH, van Mil S, Chambers J, Strautnieks S, Thompson R, Lammert F, et al. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. *Gut* 2009;58:537–544.
- [70] Meng LJ, Reyes H, Palma J, Hernandez I, Ribalta J, Sjøvall J. Profiles of bile acids and progesterone metabolites in the urine and serum of women with intrahepatic cholestasis of pregnancy. *J Hepatol* 1997;27:346–357.
- [71] Vallejo M, Briz O, Serrano MA, Monte MJ, Marin JJ. Potential role of trans-inhibition of the bile salt export pump by progesterone metabolites in the etiopathogenesis of intrahepatic cholestasis of pregnancy. *J Hepatol* 2006;44:1150–1157.
- [72] Keitel V, Vogt C, Haussinger D, Kubitz R. Combined mutations of canalicular transporter proteins cause severe intrahepatic cholestasis of pregnancy. *Gastroenterology* 2006;131:624–629.
- [73] Meier Y, Pauli-Magnus C, Zanger UM, Klein K, Schaeffeler E, Nussler AK, et al. Interindividual variability of canalicular ATP-binding-cassette (ABC)-transporter expression in human liver. *Hepatology* 2006;44:62–74.
- [74] Paulusma CC, Groen A, Kunne C, Ho-Mok KS, Spijkerboer AL, Rudi de Waart D, et al. Atp8b1 deficiency in mice reduces resistance of the canalicular membrane to hydrophobic bile salts and impairs bile salt transport. *Hepatology* 2006;44:195–204.
- [75] Ujhazy P, Ortiz D, Misra S, Li S, Moseley J, Jones H, et al. Familial intrahepatic cholestasis 1: studies of localization and function. *Hepatology* 2001;34:768–775.
- [76] Bull LN, van Eijk MJ, Pawlikowska L, DeYoung JA, Juijn JA, Liao M, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. *Nat Genet* 1998;18:219–224.
- [77] Lykavieris P, van Mil S, Cresteil D, Fabre M, Hadchouel M, Klomp L, et al. Progressive familial intrahepatic cholestasis type 1 and extrahepatic features: no catch-up of stature growth, exacerbation of diarrhea, and appearance of liver steatosis after liver transplantation. *J Hepatol* 2003;39:447–452.
- [78] Alvarez L, Jara P, Sanchez-Sabate E, Hierro L, Larrauri J, Diaz MC, et al. Reduced hepatic expression of farnesoid X receptor in hereditary cholestasis associated to mutation in ATP8B1. *Hum Mol Genet* 2004;13:2451–2460.
- [79] Chen F, Ananthanarayanan M, Emre S, Neimark E, Bull LN, Knisely AS, et al. Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity. *Gastroenterology* 2004;126:756–764.
- [80] Frankenberg T, Miloh T, Chen FY, Ananthanarayanan M, Sun AQ, Balasubramanian N, et al. The membrane protein ATPase class I type 8B member 1 signals through protein kinase C zeta to activate the farnesoid X receptor. *Hepatology* 2008;48:1896–1905.
- [81] Cai SY, Gautam S, Nguyen T, Soroka CJ, Rahner C, Boyer JL. ATP8B1 deficiency disrupts the bile canalicular membrane bilayer structure in hepatocytes, but FXR expression and activity are maintained. *Gastroenterology* 2009;136:1060–1069.
- [82] van Ooteghem NA, Klomp LW, van Berge-Henegouwen GP, Houwen RH. Benign recurrent intrahepatic cholestasis progressing to progressive familial intrahepatic cholestasis: low GGT cholestasis is a clinical continuum. *J Hepatol* 2002;36:439–443.
- [83] Painter JN, Savander M, Ropponen A, Nupponen N, Riikonen S, Ylikorkala O, et al. Sequence variation in the ATP8B1 gene and intrahepatic cholestasis of pregnancy. *Eur J Hum Genet* 2005;13:435–439.
- [84] Mullenbach R, Bennett A, Tetlow N, Patel N, Hamilton G, Cheng F, et al. ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. *Gut* 2005;54:829–834.
- [85] Keppler D, König J. Hepatic secretion of conjugated drugs and endogenous substances. *Semin Liver Dis* 2000;20:265–272.
- [86] Paulusma CC, Kool M, Bosma PJ, Scheffer GL, ter Borg F, Scheper RJ, et al. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin–Johnson syndrome. *Hepatology* 1997;25:1539–1542.
- [87] Sookoian S, Castano G, Burgueno A, Gianotti TF, Pirola CJ. Association of the multidrug-resistance-associated protein gene (ABCC2) variants with intrahepatic cholestasis of pregnancy. *J Hepatol* 2008;48:125–132.
- [88] Prieto J, Qian C, Garcia N, Diez J, Medina JF. Abnormal expression of anion exchanger genes in primary biliary cirrhosis. *Gastroenterology* 1993;105:572–578.
- [89] Medina JF, Martinez A, Vazquez JJ, Prieto J. Decreased anion exchanger 2 immunoreactivity in the liver of patients with primary biliary cirrhosis. *Hepatology* 1997;25:12–17.
- [90] Prieto J, Garcia N, Marti-Climent JM, Penuelas I, Richter JA, Medina JF. Assessment of biliary bicarbonate secretion in humans by positron emission tomography. *Gastroenterology* 1999;117:167–172.
- [91] Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, et al. Ae2a,b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. *Gastroenterology* 2008;134:1482–1493.
- [92] Fickert P, Trauner M. When lightning strikes twice: the plot thickens for a dual role of the anion exchanger 2 (AE2/SLC4A2) in the pathogenesis and treatment of primary biliary cirrhosis. *J Hepatol* 2009;50:633–635.
- [93] Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066–1073.
- [94] Feranchak AP, Sokol RJ. Cholangiocyte biology and cystic fibrosis liver disease. *Semin Liver Dis* 2001;21:471–488.
- [95] Blanco PG, Zaman MM, Junaidi O, Sheth S, Yantiss RK, Nasser IA, et al. Induction of colitis in cfr^{-/-} mice results in bile duct injury. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G491–G496.
- [96] Sheth S, Shea JC, Bishop MD, Chopra S, Regan MM, Malmberg E, et al. Increased prevalence of CFTR mutations and variants and decreased chloride secretion in primary sclerosing cholangitis. *Hum Genet* 2003;113:286–292.
- [97] Pall H, Zielinski J, Jonas MM, DaSilva DA, Potvin KM, Yuan XW, et al. Primary sclerosing cholangitis in childhood is associated with abnormalities in cystic fibrosis-mediated chloride channel function. *J Pediatr* 2007;151:255–259.
- [98] McGill JM, Williams DM, Hunt CM. Survey of cystic fibrosis transmembrane conductance regulator genotypes in primary sclerosing cholangitis. *Dig Dis Sci* 1996;41:540–542.
- [99] Henckaerts L, Jaspers M, Van Steenberghe W, Vliegen L, Fevery J, Nuytten H, et al. Cystic fibrosis transmembrane conductance regulator gene polymorphisms in patients with primary sclerosing cholangitis. *J Hepatol* 2009;50:150–157.

- [100] Carlton VE, Harris BZ, Puffenberger EG, Batta AK, Knisely AS, Robinson DL, et al. Complex inheritance of familial hypercholelanemia with associated mutations in TJP2 and BAAT. *Nat Genet* 2003;34:91–96.
- [101] Hadj-Rabia S, Baala L, Vabres P, Hamel-Teillac D, Jacquemin E, Fabre M, et al. Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. *Gastroenterology* 2004;127:1386–1390.
- [102] Carlton VE, Pawlikowska L, Bull LN. Molecular basis of intrahepatic cholestasis. *Ann Med* 2004;36:606–617.
- [103] Sundaram SS, Alonso EM, Anand R. Outcomes after liver transplantation in young infants. *J Pediatr Gastroenterol Nutr* 2008;47:486–492.
- [104] Van Mil SW, Milona A, Dixon PH, Mullenbach R, Geenes VL, Chambers J, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. *Gastroenterology* 2007;133:507–516.
- [105] Kovacs P, Kress R, Rocha J, Kurtz U, Miquel JF, Nervi F, et al. Variation of the gene encoding the nuclear bile salt receptor FXR and gallstone susceptibility in mice and humans. *J Hepatol* 2008;48:116–124.
- [106] Karlsen TH, Lie BA, Frey Frosle K, Thorsby E, Broome U, Schrupf E, et al. Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. *Gastroenterology* 2006;131:781–787.
- [107] Lee J, Boyer JL. Molecular alterations in hepatocyte transport mechanisms in acquired cholestatic liver disorders. *Semin Liver Dis* 2000;20:373–384.
- [108] Trauner M, Wagner M, Fickert P, Zollner G. Molecular regulation of hepatobiliary transport systems: clinical implications for understanding and treating cholestasis. *J Clin Gastroenterol* 2005;39:S111–S124.
- [109] Plebani M, Panozzo MP, Basso D, De Paoli M, Biasin R, Infantolino D. Cytokines and the progression of liver damage in experimental bile duct ligation. *Clin Exp Pharmacol Physiol* 1999;26:358–363.
- [110] Paumgartner G, Pusch T. Medical treatment of cholestatic liver disease. *Clin Liver Dis* 2008;12:53–80.
- [111] Zollner G, Wagner M, Fickert P, Silbert D, Gumhold J, Zatloukal K, et al. Expression of bile acid synthesis and detoxification enzymes and the alternative bile acid efflux pump MRP4 in patients with primary biliary cirrhosis. *Liver Int* 2007;27:920–929.
- [112] Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. *Biochem Biophys Res Commun* 2002;293:145–149.
- [113] Fang HL, Strom SC, Cai H, Falany CN, Kocarek TA, Runge-Morris M. Regulation of human hepatic hydroxysteroid sulfotransferase gene expression by the peroxisome proliferator-activated receptor alpha transcription factor. *Mol Pharmacol* 2005;67:1257–1267.
- [114] Gartung C, Ananthanarayanan M, Rahman MA, Schuele S, Nundy S, Soroka CJ, et al. Down-regulation of expression and function of the rat liver Na⁺/bile acid cotransporter in extrahepatic cholestasis. *Gastroenterology* 1996;110:199–209.
- [115] Jung D, Hagenbuch B, Fried M, Meier PJ, Kullak-Ublick GA. Role of liver-enriched transcription factors and nuclear receptors in regulating the human, mouse, and rat Ntcp gene. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G752–G761.
- [116] Zollner G, Wagner M, Fickert P, Geier A, Fuchsbichler A, Silbert D, et al. Role of nuclear receptors and hepatocyte-enriched transcription factors for Ntcp repression in biliary obstruction in mouse liver. *Am J Physiol Gastrointest Liver Physiol* 2005;289:G798–G805.
- [117] Park YJ, Qatanani M, Chua SS, LaRey JL, Johnson SA, Watanabe M, et al. Loss of orphan receptor small heterodimer partner sensitizes mice to liver injury from obstructive cholestasis. *Hepatology* 2008;47:1578–1586.
- [118] Jung D, Kullak-Ublick GA. Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. *Hepatology* 2003;37:622–631.
- [119] Lee JM, Trauner M, Soroka CJ, Stieger B, Meier PJ, Boyer JL. Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. *Gastroenterology* 2000;118:163–172.
- [120] Wagner M, Fickert P, Zollner G, Fuchsbichler A, Silbert D, Tsybrovskyy O, et al. Role of farnesoid X receptor in determining hepatic ABC transporter expression and liver injury in bile duct-ligated mice. *Gastroenterology* 2003;125:825–838.
- [121] Stedman C, Liddle C, Coulter S, Sonoda J, Alvarez JG, Evans RM, et al. Benefit of farnesoid X receptor inhibition in obstructive cholestasis. *Proc Natl Acad Sci USA* 2006;103:11323–11328.
- [122] Maher JM, Dieter MZ, Aleksunes LM, Slitt AL, Guo G, Tanaka Y, et al. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear factor-E2-related factor-2 transcriptional pathway. *Hepatology* 2007;46:1597–1610.
- [123] Okada K, Shoda J, Taguchi K, Maher JM, Ishizaki K, Inoue Y, et al. Ursodeoxycholic acid stimulates Nrf2-mediated hepatocellular transport, detoxification, and antioxidative stress systems in mice. *Am J Physiol Gastrointest Liver Physiol* 2008;295:G735–G747.
- [124] Wagner M, Halilbasic E, Marschall HU, Zollner G, Fickert P, Langner C, et al. CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice. *Hepatology* 2005;42:420–430.
- [125] Zelcer N, van de Wetering K, de Waart R, Scheffer GL, Marschall HU, Wielinga PR, et al. Mice lacking Mrp3 (Abcc3) have normal bile salt transport, but altered hepatic transport of endogenous glucuronides. *J Hepatol* 2006;44:768–775.
- [126] Mennone A, Soroka CJ, Cai SY, Harry K, Adachi M, Hagey L, et al. Mrp4^{-/-} mice have an impaired cytoprotective response in obstructive cholestasis. *Hepatology* 2006;43:1013–1021.
- [127] Boyer JL, Trauner M, Mennone A, Soroka CJ, Cai SY, Moustafa T, et al. Upregulation of a basolateral FXR-dependent bile acid efflux transporter OSTalpha-OSTbeta in cholestasis in humans and rodents. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G1124–G1130.
- [128] Gustafsson J. Effect of biliary obstruction on 26-hydroxylation of C27-steroids in bile acid synthesis. *J Lipid Res* 1978;19:237–243.
- [129] Dueland S, Reichen J, Everson GT, Davis RA. Regulation of cholesterol and bile acid homeostasis in bile-obstructed rats. *Biochem J* 1991;280:373–377.
- [130] Pandak WM, Heuman DM, Hylemon PB, Chiang JY, Vlahcevic ZR. Failure of intravenous infusion of taurocholate to down-regulate cholesterol 7 alpha-hydroxylase in rats with biliary fistulas. *Gastroenterology* 1995;108:533–544.
- [131] Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005;2:217–225.
- [132] Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models. *J Clin Invest* 2003;112:1678–1687.
- [133] Schaap FG, van der Gaag NA, Gouma DJ, Jansen PL. High expression of the bile salt-homeostatic hormone fibroblast growth factor 19 in the liver of patients with extrahepatic cholestasis. *Hepatology* 2008;49:1228–1235.

- [134] Bertolotti M, Carulli L, Concari M, Martella P, Loria P, Tagliafico E, et al. Suppression of bile acid synthesis, but not of hepatic cholesterol 7 α -hydroxylase expression, by obstructive cholestasis in humans. *Hepatology* 2001;34:234–242.
- [135] Oswald M, Kullak-Ublick GA, Paumgartner G, Beuers U. Expression of hepatic transporters OATP-C and MRP2 in primary sclerosing cholangitis. *Liver* 2001;21:247–253.
- [136] Shoda J, Kano M, Oda K, Kamiya J, Nimura Y, Suzuki H, et al. The expression levels of plasma membrane transporters in the cholestatic liver of patients undergoing biliary drainage and their association with the impairment of biliary secretory function. *Am J Gastroenterol* 2001;96:3368–3378.
- [137] Floch MH, Gershengoren W, Elliott S, Spiro HM. Bile acid inhibition of the intestinal microflora – a function for simple bile acids. *Gastroenterology* 1971;61:228–233.
- [138] Lorenzo-Zuniga V, Bartoli R, Planas R, Hofmann AF, Vinado B, Hagey LR, et al. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. *Hepatology* 2003;37:551–557.
- [139] Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci USA* 2006;103:3920–3925.
- [140] Trauner M, Fickert P, Stauber RE. Inflammation-induced cholestasis. *J Gastroenterol Hepatol* 1999;14:946–959.
- [141] Geier A, Fickert P, Trauner M. Mechanisms of disease: mechanisms and clinical implications of cholestasis in sepsis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:574–585.
- [142] Luster MI, Germolec DR, Yoshida T, Kayama F, Thompson M. Endotoxin-induced cytokine gene expression and excretion in the liver. *Hepatology* 1994;19:480–488.
- [143] Sewnath ME, Van Der Poll T, Van Noorden CJ, Ten Kate FJ, Gouma DJ. Endogenous interferon gamma protects against cholestatic liver injury in mice. *Hepatology* 2002;36:1466–1477.
- [144] Ohlinger W, Dinges HP, Zatloukal K, Mair S, Gollowitsch F, Denk H. Immunohistochemical detection of tumor necrosis factor- α , other cytokines and adhesion molecules in human livers with alcoholic hepatitis. *Virchows Arch A Pathol Anat Histopathol* 1993;423:169–176.
- [145] Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 2008;48:1632–1643.
- [146] Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest* 2006;116:2280–2289.
- [147] Bolder U, Ton-Nu HT, Scheingart CD, Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. *Gastroenterology* 1997;112:214–225.
- [148] Moseley RH, Wang W, Takeda H, Lown K, Shick L, Ananthanarayanan M, et al. Effect of endotoxin on bile acid transport in rat liver: a potential model for sepsis-associated cholestasis. *Am J Physiol* 1996;271:G137–G146.
- [149] Trauner M, Arrese M, Lee H, Boyer JL, Karpen SJ. Endotoxin downregulates rat hepatic ntcp gene expression via decreased activity of critical transcription factors. *J Clin Invest* 1998;101:2092–2100.
- [150] Green RM, Beier D, Gollan JL. Regulation of hepatocyte bile salt transporters by endotoxin and inflammatory cytokines in rodents. *Gastroenterology* 1996;111:193–198.
- [151] Zollner G, Fickert P, Silbert D, Fuchsichler A, Stumtner C, Zatloukal K, et al. Induction of short heterodimer partner 1 precedes downregulation of Ntcp in bile duct-ligated mice. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G184–G191.
- [152] Trauner M, Arrese M, Soroka CJ, Ananthanarayanan M, Koepffel TA, Schlosser SF, et al. The rat canalicular conjugate export pump (Mrp2) is down-regulated in intrahepatic and obstructive cholestasis. *Gastroenterology* 1997;113:255–264.
- [153] Gerloff T, Geier A, Roots I, Meier PJ, Gartung C. Functional analysis of the rat bile salt export pump gene promoter. *Eur J Biochem* 2002;269:3495–3503.
- [154] Denson LA, Auld KL, Schiek DS, McClure MH, Mangelsdorf DJ, Karpen SJ. Interleukin-1 β suppresses retinoid transactivation of two hepatic transporter genes involved in bile formation. *J Biol Chem* 2000;275:8835–8843.
- [155] Li D, Zimmerman TL, Thevananther S, Lee HY, Kurie JM, Karpen SJ. Interleukin-1 β mediated suppression of RXR:RAR transactivation of the Ntcp promoter is JNK-dependent. *J Biol Chem* 2002;277:31416–31422.
- [156] Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular re-localization of hepatic RXR α : a novel mechanism for reduced hepatic gene expression in inflammation. *Nucl Recept* 2004;2:4.
- [157] Kim MS, Shigenaga J, Moser A, Feingold K, Grunfeld C. Repression of farnesoid X receptor during the acute phase response. *J Biol Chem* 2003;278:8988–8995.
- [158] Teng S, Piquette-Miller M. The involvement of the pregnane X receptor in hepatic gene regulation during inflammation in mice. *J Pharmacol Exp Ther* 2005;312:841–848.
- [159] Elferink MG, Olinga P, Draaisma AL, Merema MT, Faber KN, Slooff MJ, et al. LPS-induced downregulation of MRP2 and BSEP in human liver is due to a posttranscriptional process. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G1008–G1016.
- [160] Zollner G, Fickert P, Zenz R, Fuchsichler A, Stumtner C, Kenner L, et al. Hepatobiliary transporter expression in percutaneous liver biopsies of patients with cholestatic liver diseases. *Hepatology* 2001;33:633–646.
- [161] Kubitz R, Wettstein M, Warskulat U, Haussinger D. Regulation of the multidrug resistance protein 2 in the rat liver by lipopolysaccharide and dexamethasone. *Gastroenterology* 1999;116:401–410.
- [162] Teng S, Piquette-Miller M. Regulation of transporters by nuclear hormone receptors: implications during inflammation. *Mol Pharm* 2008;5:67–76.
- [163] Keitel V, Donner M, Winandy S, Kubitz R, Haussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells. *Biochem Biophys Res Commun* 2008;372:78–84.
- [164] Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 2003;278:9435–9440.
- [165] Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. Drug- and estrogen-induced cholestasis through inhibition of the paepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology* 2000;118:422–430.
- [166] Fattinger K, Funk C, Pantze M, Weber C, Reichen J, Stieger B, et al. The endothelin antagonist bosentan inhibits the canalicular bile salt export pump: a potential mechanism for hepatic adverse reactions. *Clin Pharmacol Ther* 2001;69:223–231.
- [167] Funk C, Ponelle C, Scheuermann G, Pantze M. Cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity: in vivo and in vitro interaction at the canalicular bile salt export pump (Bsep) in the rat. *Mol Pharmacol* 2001;59:627–635.
- [168] Bode KA, Donner MG, Leier I, Keppler D. Inhibition of transport across the hepatocyte canalicular membrane by the antibiotic fusidate. *Biochem Pharmacol* 2002;64:151–158.
- [169] Marshall HU, Wagner M, Zollner G, Trauner M. Clinical hepatotoxicity. Regulation and treatment with inducers of transport and cofactors. *Mol Pharm* 2007;4:895–910.
- [170] Marshall HU, Wagner M, Zollner G, Fickert P, Diczfalusy U, Gumhold J, et al. Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. *Gastroenterology* 2005;129:476–485.

- [171] Zollner G, Fickert P, Fuchsbichler A, Silbert D, Wagner M, Arbeiter S, et al. Role of nuclear bile acid receptor, FXR, in adaptive ABC transporter regulation by cholic and ursodeoxycholic acid in mouse liver, kidney and intestine. *J Hepatol* 2003;39:480–488.
- [172] Zollner G, Fickert P, Silbert D, Fuchsbichler A, Marschall HU, Zatloukal K, et al. Adaptive changes in hepatobiliary transporter expression in primary biliary cirrhosis. *J Hepatol* 2003;38:717–727.
- [173] Beuers U. Drug insight: mechanisms and sites of action of ursodeoxycholic acid in cholestasis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:318–328.
- [174] Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic acid. *Gastroenterology* 2006;130:715–720.
- [175] Corpechot C, Abenavoli L, Rabahi N, Chretien Y, Andreani T, Johanet C, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology* 2008;48:871–877.
- [176] Cullen SN, Chapman RW. The medical management of primary sclerosing cholangitis. *Semin Liver Dis* 2006;26:52–61.
- [177] Lindor KD. Ursodiol for primary sclerosing cholangitis. Mayo Primary Sclerosing Cholangitis-Ursodeoxycholic Acid Study Group. *N Engl J Med* 1997;336:691–695.
- [178] Lindor KD, Enders FB, Schmoll JA, Hoskin TL, Jorgensen RA, Petz JL, et al. Randomized, double-blind controlled trial of high-dose ursodeoxycholic acid (UDCA) for primary sclerosing cholangitis. *Hepatology* 2008;48:94A, LB92.
- [179] Claudel T, Sturm E, Kuipers F, Staels B. The farnesoid X receptor: a novel drug target? *Expert Opin Investig Drugs* 2004;13:1135–1148.
- [180] Huang L, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR, et al. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. *J Biol Chem* 2003;278:51085–51090.
- [181] Moschetta A, Bookout AL, Mangelsdorf DJ. Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. *Nat Med* 2004;10:1352–1358.
- [182] Fickert P, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Zatloukal K, et al. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2006;130:465–481.
- [183] Yoon YB, Hagey LR, Hofmann AF, Gurantz D, Michelotti EL, Steinbach JH. Effect of side-chain shortening on the physiologic properties of bile acids: hepatic transport and effect on biliary secretion of 23-nor-ursodeoxycholate in rodents. *Gastroenterology* 1986;90:837–852.
- [184] Halilbasic E, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, et al. Side chain structure determines unique physiologic and therapeutic properties of norUrsodeoxycholic acid in Mdr2^{-/-} mice. *Hepatology* 2009;49:1972–1981.
- [185] Bachs L, Pares A, Elena M, Piera C, Rodes J. Effects of long-term rifampicin administration in primary biliary cirrhosis. *Gastroenterology* 1992;102:2077–2080.
- [186] Stiehl A, Thaler MM, Admirand WH. The effects of phenobarbital on bile salts and bilirubin in patients with intrahepatic and extrahepatic cholestasis. *N Engl J Med* 1972;286:858–861.
- [187] Nakai S, Masaki T, Kurokohchi K, Deguchi A, Nishioka M. Combination therapy of bezafibrate and ursodeoxycholic acid in primary biliary cirrhosis: a preliminary study. *Am J Gastroenterol* 2000;95:326–327.
- [188] Kanda T, Yokosuka O, Imazeki F, Saisho H. Bezafibrate treatment: a new medical approach for PBC patients? *J Gastroenterol* 2003;38:573–578.
- [189] Ritzel U, Leonhardt U, Nather M, Schafer G, Armstrong VW, Ramadori G. Simvastatin in primary biliary cirrhosis: effects on serum lipids and distinct disease markers. *J Hepatol* 2002;36:454–458.
- [190] Hooiveld GJ, Vos TA, Scheffer GL, Van Goor H, Koning H, Bloks V, et al. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) induce hepatic expression of the phospholipid translocase mdr2 in rats. *Gastroenterology* 1999;117:678–687.
- [191] Kok T, Bloks VW, Wolters H, Havinga R, Jansen PL, Staels B, et al. Peroxisome proliferator-activated receptor alpha (PPAR-alpha)-mediated regulation of multidrug resistance 2 (Mdr2) expression and function in mice. *Biochem J* 2003;369:539–547.